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Vegge, Christina Skovgaard; Brøndsted, Lone; Ingmer, Hanne

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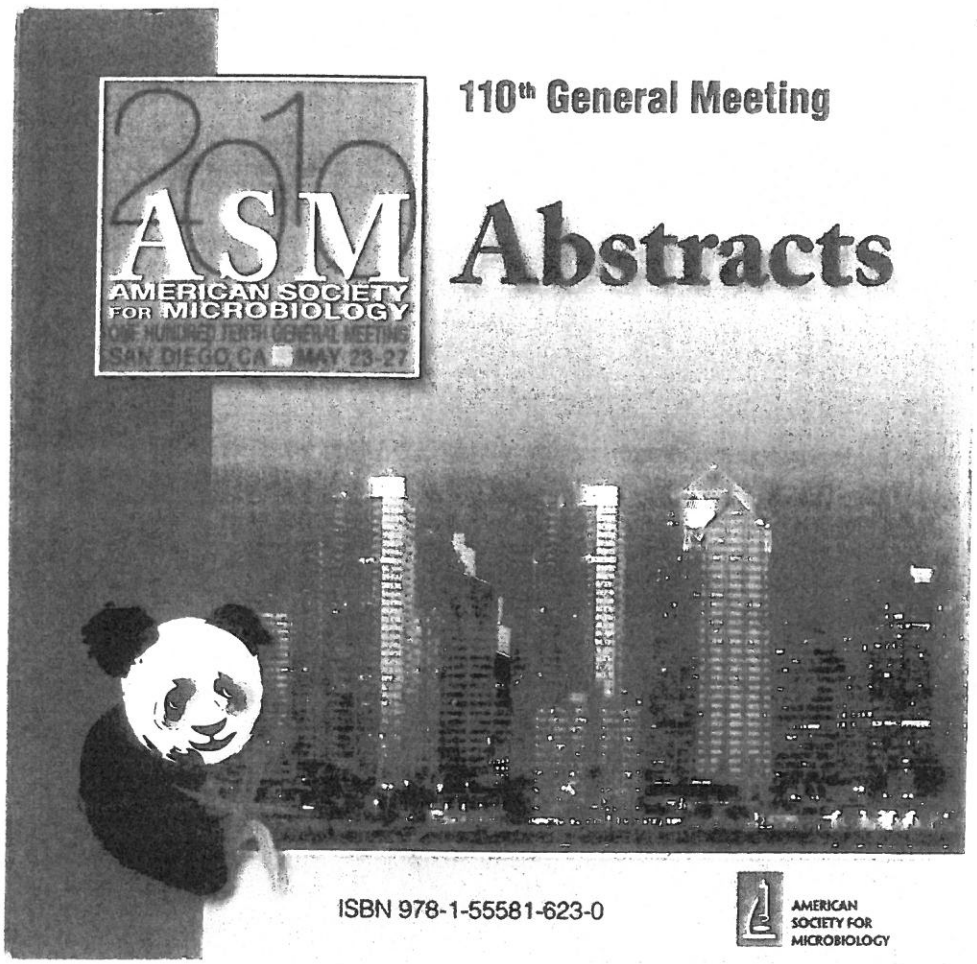

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Abstracts

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UNIVERSITY OF COPENHAGEN

Campylobacter jejuni Competence Exceeds Growth

Christina S. Vegge, Lone Brøndsted and Hanne Ingmer

Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark

Contact information: Stigboejlen 4, 1870 Frederiksberg C, Denmark
Phone: +45 35 33 27 62
E-mail: csv@life.ku.dk

Introduction

The Gram-negative organism *Campylobacter jejuni* is the primary food borne bacterial pathogen in the developed world causing millions of gastroenteritis cases each year. *C. jejuni* is a fastidious organism, as it only grows under strictly microaerophilic conditions in a narrow temperature interval of 30-46° C. It is well recognized that *C. jejuni* lacks several classical stress response mechanisms resulting in hampered stress adaptation and *C. jejuni* is therefore often described as a sensitive organism. In contrast, horizontal gene transfer occurs very efficiently as *C. jejuni* is naturally competent, i.e. the bacterium can take up extracellular DNA from the environment, and incorporate the DNA into its own genome by homologous recombination. In contrast to the well studied mechanisms of natural competence in e.g. *Bacillus subtilis*, *Streptococcus* and *Neisseria gonorrheae* the mechanism and regulation of competence in *C. jejuni* is largely unknown.

Aim of study

To explore the environmental conditions supporting natural competence of *C. jejuni* NCTC11168 and investigate the potential regulation of this mechanism.

Methods

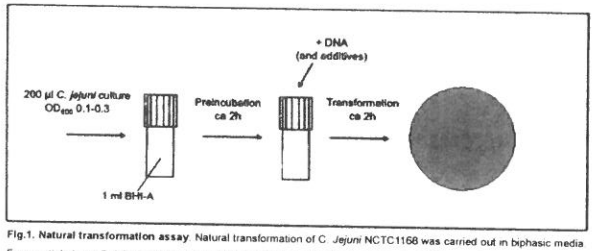

Fig.1. Natural transformation assay. Natural transformation of C. *Jejuni* NCTC1168 was carried out in biphasic media. Exponential phase *C. jejuni* cultivated in BHI was added to a cryotube containing 1 ml BHI-agar. Following two hours of preincubation, the DNA was added and the transformation allowed to proceed for two hours before plating on selective Base II agar. Thee assay was carried out at 37° C under microaerobic conditions and with isogenic chromosomal DNA carrying antibiotic resistant markers unless otherwise described.

Results

Natural transformation at both growth permissive and restrictive conditions

With the objective of investigating the regulation of competence in *C. jejuni* we sought to identify environmental conditions restrictive for natural transformation. Firstly, we explored natural transformation at different growth phases and discovered this bacterium to be competent from early exponential phase to late stationary phase, but the transformation efficiency is far superior in the exponential phase (results not shown). Furthermore, transformation assays were carried out under several growth permissive and restrictive conditions. The DNA uptake was most efficient at the optimal growth conditions but to our surprise, we observed **C. *jejuni* takes up DNA by natural transformation at various conditions both permissive and restrictive for growth** (Table 1 and Fig. 2). These results indicate the competence of *C. jejuni* is not strictly regulated but simply central for this organism, since competence seems to exceed growth. This is in strong contrast to the highly controlled mechanisms of competence in *B. subtilis*, *Streptococcus* and *N. Gonorrheae*.

Temperature	Aerobic	Microaerophilic	Anaerobic
5° C	0.2	0	0
20° C	0.2	0	0
30° C	5	0.2	0.1
37° C	5-50	Index 100	5
42° C	20-50	20-50	2

Table 1. Natural transformation at growth-restrictive conditions of temperature and oxygen level. *C. jejuni* only grows at microaerophilic conditions from 30-42° C (grey area), but natural transformation also takes place at both aerobic and anaerobic conditions and at temperatures not supporting growth (red/brown area). The level of transformation is stated relative to results obtained at microaerophilic, 37° C.

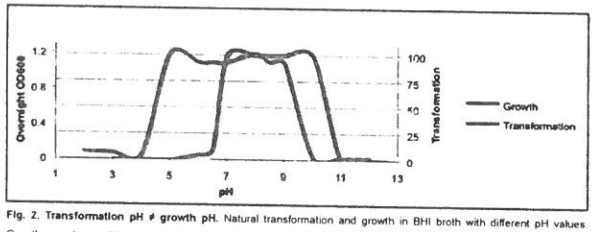

Fig. 2. Transformation pH ≠ growth pH. Natural transformation and growth in BHI broth with different pH values. Growth was observed in the pH interval 5-9 (grey curve), while natural transformation takes place at pH 6-10 (red curve). The level of transformation is stated relative to the result obtained at pH 7.

Cellular processes and parameters important for DNA uptake

To identify parameters and cellular processes of importance for DNA uptake, the transformation assay was carried out with additives inhibiting different molecular mechanisms and with various forms of DNA. These assays showed that **protein synthesis and energy production by oxidative phosphorylation are very important for transformation**, while extracellular proteins are not relevant for this mechanism (Table 2). In addition, the DNA uptake appears to be restricted to chromosomal DNA of *C. jejuni* origin, since the source of DNA had a paramount effect on the transformation efficiency (Table 3). It was not possible to evaluate the effect of transcriptional inhibition by rifampicin, since *C. jejuni* NCTC11168 is resistant to this antibiotic.

Additive	Inhibition	Effect on survival	Transformation
Chloramphenicol	Protein synthesis	43-70% reduction	0
Rifampicin	Transcription	0 - Resistant!	100
HQNO	Respiration	50%	2
Sodium azide	Respiration	50%	5
Trypsin	Extracellular proteins	0%	100
Proteinase K	Extracellular proteins	0%	100

Table 2. Protein synthesis and respiration important for natural transformation. Transformation assays were carried out with inhibitors of cellular mechanisms. The effect of the additives on survival and level of transformation is stated relative to a control without inhibitor.

Type of DNA	Cam^R transformants / DNA molecule
Isogenic chromosomal DNA	10^{-2}
PCR fragment	10^{-12}
Plasmid (suicide)	10^{-11}
Helicobacter pylori or *Arcobacter butzleri* chromosomal DNA	0

Table 3. DNA uptake restricted to chromosomal DNA of *C. jejuni* origin. Natural transformation of different forms of DNA carrying isogenic sequences flanking a Cam^R gene. Uptake of chromosomal DNA from *Helicobacter pylori* and *Arcobacter butzleri* was tested in a competition assay with isogenic chromosomal DNA.

Conclusions

- **Natural transformation of *C. jejuni* takes place at growth restrictive conditions**
- **Competence of *C. jejuni* does not seem to be strictly regulated**
- **Protein synthesis and oxidative phosphorylation are important for transformation**
- **DNA uptake is restricted to chromosomal DNA of *C. jejuni* origin**